

Personality and parasites: sex-dependent associations between avian malaria infection and multiple behavioural traits

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Abstract The evolution and ecology of consistent behavioural variation, or personality, is currently the focus of much attention in natural populations. Associations between personality traits and parasite infections are increasingly being reported, but the extent to which multiple behavioural traits might be associated with parasitism at the same time is largely unknown. Here, we use a population of great tits, *Parus major*, to examine whether infection by avian malaria (*Plasmodium* and *Leucocytozoon*) is associated with three behavioural traits assayed under standardized conditions. All of these traits are of broad ecological significance and two of them are repeatable or heritable in our population. Here, we show weak correlations between some but not all of these behavioural traits, and sex-

dependent associations between all three behavioural traits and parasite infection. Infected males showed increased problem-solving performance whereas infected females showed reduced performance; furthermore, uninfected females were four times more likely to solve problems than uninfected males. Infected females were more exploratory than uninfected females, but infection had no effect on males. Finally, infected males were more risk-averse than uninfected males but females were unaffected. Our results demonstrate the potential for complex interactions between consistent personality variation and parasite infection, though we discuss the difficulty of attributing causality in these associations. Accounting for complex parasite-behaviour associations may prove essential in understanding the evolutionary ecology of behavioural variation and the dynamics of host–parasite interactions.

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Introduction

Personalities or behavioural syndromes have been described across a wide range of taxa (Gosling and John 1999; Sih et al. 2004b) and can be defined as consistent behavioural differences between individuals. These differences can take the form of behavioural correlations within the same trait across the same or different contexts or correlations between different behavioural traits (Gosling and John 1999; Sih et al. 2004b; Réale et al. 2007). Personality variation is currently the focus of much research, especially in the context of a range of traits that can be easily measured under standardized conditions, for

example exploration behaviour, aggressiveness, boldness, or sociability. One reason for this focus is that these traits effectively represent personality axes of variation because they predict behaviour in a whole range of other traits, and therefore have major consequences for a variety of ecological and evolutionary processes (Sih et al. 2004a; Réale et al. 2007), including susceptibility to parasitism (Barber and Dingemanse 2010; Boyer et al. 2010).

Parasites are ubiquitous in animal populations and links between parasite infection and host behaviour can arise through a variety of mechanisms (Moore 2002; Lefèvre and Thomas 2008; Lefèvre et al. 2009). Altering host behaviour may be adaptive for trophically transmitted parasites by increasing the likelihood of transmission (Sanchez et al. 2008) but can also arise as a by-product of the diversion of host energy reserves to parasites (Edelaar et al. 2003), or as a side effect of non-target host infection (Webster 2001; Lefèvre et al. 2008). It is also well documented that the threat of parasitism can generate avoidance behaviours that would otherwise not be observed (Hart 1990). Finally, numerous studies suggest that a range of behavioural traits may make individuals more susceptible to directly, vector- and trophically transmitted parasites (Wilson et al. 1993; Moore 2002; Barber and Dingemanse 2010; Boyer et al. 2010). Several challenges face these latter studies. The first is the possibility that parasitism was the underlying cause, not consequence, of the behavioural variation. Secondly, it is rarely known whether these traits are consistent within individuals and therefore the adaptive significance of these associations remain unclear. Thirdly, studies that have examined behavioural associations with parasitism have tended to focus on one specific behavioural trait rather than on examining associations with a suite of behaviours (Valkiunas 2005; Holmstad et al. 2006). It therefore remains unclear how general the associations between parasitism and behaviour are likely to be within individuals, and predictions will differ depending on the nature of transmission of the parasite. Finally, the extent to which these associations are sex dependent are not well understood. Numerous mechanisms could lead to sex differences in the association between infection and behaviour. For example, sex differences in behavioural traits associated with latent toxoplasmosis in humans, where effects occur as a side effect of non-target host infection, are thought to be mediated by the alteration of dopamine levels (Lindová et al. 2006; Flegr et al. 2008), which counteract symptoms among infected women (Lindová et al. 2006). Testosterone suppresses the immune system (Oppliger et al. 2004), and male birds with higher plasma testosterone levels tend to have a higher intensity of avian malaria infection (Saino et al. 1995). The sexes can also differ in their behaviour, both during and outside the breeding season (Gosler 1987) which may lead to a differential likelihood of infection.

Avian malaria is an extremely widespread, vector-transmitted parasite amongst birds (Valkiunas 2005) and can have lethal and sub-lethal effects on both individuals and populations (Van Riper et al. 1986). Detrimental associations have been found between avian malaria infection and many life-history traits (e.g. Dufva 1996; Merino et al. 2000; Møller et al. 2004; Knowles et al. 2010), although the mechanisms underlying these associations are unknown. These associations may be sex specific: for example, Møller et al. (2004) found infection by malaria parasites to delay arrival date in male swallows but not females. However, little is known about the sex-specific nature of associations between avian malaria and behaviour, as many studies focus on the effects of infection on one sex (Dufva 1996; Merino et al. 2000; Knowles et al. 2010).

In this paper, we use a natural population of a generalist passerine bird, the great tit *Parus major*, to examine associations between malaria infection and three contrasting behavioural traits. Two malaria parasite families, *Plasmodium* and *Leucocytozoon*, are known to be present at high prevalence within parts of our study population (Wood et al. 2007; Cosgrove et al. 2008) and reach their highest prevalence in autumn (Cosgrove et al. 2008). A previous study experimentally demonstrated that *Plasmodium* infection has a direct and substantial effect on female reproductive fitness in blue tits, *Cyanistes caeruleus* (Knowles et al. 2010), confirming that functionally significant effects of malaria on behaviour in great tits are plausible.

The behavioural traits we focus on—exploration behaviour of a novel environment, problem-solving performance and startle latency—were chosen because they are easily measured under standardized conditions (van Oers et al. 2004; Quinn et al. 2009; Cole et al. 2011), they have broad ecological significance (Greenberg 2003; Réale et al. 2007) and because they have been associated with parasite infection in other systems (Holmstad et al. 2006; Garamzegi et al. 2007; Boyer et al. 2010). Highly exploratory individuals may be more likely to become infected (Wilson et al. 1993) because host activity level is known to increase susceptibility to parasitism generally (Boyer et al. 2010). Malaria prevalence varies spatially in our system (Wood et al. 2007), so more exploratory individuals may be more likely to come across the vectors of avian malaria and become infected. Performance in novel problem-solving tasks is thought to provide a measure of general cognitive ability (Roth and Dicke 2005) and innovativeness (Laland and Reader 1999; Webster and Lefebvre 2001). Because innovative foraging behaviour has been associated with avian malaria infection across species (Garamzegi et al. 2007), we also expected that individual variation in problem-solving performance might be linked to malaria infection. Latency of response to a startle stimulus is a measure of risk-taking or anti-predation behaviour, as not

responding to a sudden movement by fleeing increases the likelihood of falling prey to a predator. Infection by malaria parasites has been associated with an increased likelihood of freezing, as opposed to fleeing, in both chaffinches (Valkiunas 2005) and willow ptarmigan (Holmstad et al. 2006) but conversely, in a trophically transmitted acanthocephalan parasite system, it has also been shown to increase escape behaviour (Médoc et al. 2009).

Our aim was to investigate the extent to which *Plasmodium* and *Leucocytozoon* infections are associated with variation in exploration behaviour, problem-solving performance and startle response simultaneously among wild-caught birds temporarily taken into captivity. Exploration behaviour and problem-solving performance are also the subject of intense study in our population; elsewhere we demonstrate that both are repeatable and the former is heritable (Quinn et al. 2009; Cole et al. 2011). Due to the potentially diverse ways in which associations between parasitism and behaviour may arise, we did not make any a priori predictions regarding the direction of effects we expected. However, we expected differences between the sexes because of hormonally mediated variation observed in other species (Lindová et al. 2006; Flegr et al. 2008) and because the sexes intrinsically differ in their behaviour during reproduction which could lead to differences in exposure to vectors. We show sex differences in associations between malaria infection and each behavioural trait and discuss whether direction of causality can be inferred from the patterns observed.

Materials and methods

Study site and catching procedure

Great tits were captured in mist nets at feeding stations within Wytham Woods, Oxford, UK, during October 2008 and 2009. We chose this period because malaria peaks in detectability at this specific time of the year and shows low prevalence for much of the rest of the year (Cosgrove et al. 2008). Following capture, birds were aged and sexed according to plumage characteristics (Svensson 1992), weighed, and all unringed individuals were fitted with a unique BTO metal leg ring. They were then taken into captivity at Wytham Field station, approximately 2 km from the capture site. Birds were housed as per Quinn et al. (2009) for no more than 48 h and were blood-sampled by venipuncture of the brachial vein just before release.

Behavioural assays

At 17:00 h on the day of capture, a problem-solving task, consisting of a vertical transparent Perspex tube containing

a platform supported by a horizontal lever, was presented to each bird. The device was baited with peanuts placed on the platform. To solve the task, birds had to remove the lever from the device, causing the platform to drop and the peanuts to fall into a feeding dish positioned below the device (Cole et al. 2011). When the task was set, a single peanut was placed in this feeding dish to attract the bird to the device. Birds were not food deprived during this trial; however, the food reward in the device was preferred to the standard food available in the cage (E. Cole, personal observation). The device was then left overnight and at 08:00 h the following morning, whether or not the bird had solved the problem (defined as removing the lever either completely, or sufficiently to release the bait) was recorded and used as the response variable. The likelihood of solving this task is unrelated to activity level per se and represents individual differences in the ability to forage innovatively (Cole et al. 2011).

The exploration behaviour (EB) of each bird was assessed between 08:00 and 12:00 during the morning following capture, using methods described more fully in Quinn et al. (2009), differing only in that the assay duration was shortened from 8 to 2 min. Previous work has shown the 2- and 8-min assays to be highly correlated (Quinn et al. 2009). An assay commenced 20 s after a bird was first coaxed from its cage through a trapdoor leading into an adjoining novel environment room and lasted for 2 min, after which the bird was coaxed back into its cage. The assay room was based on Verbeek et al. (1994). The frequency and location of all movements were recorded using a handheld event recorder (Psion Workabout, with Observer mobile software, Noldus Information Technology, Nottingham, UK), generating 12 behavioural measures (see Appendix S1 of Quinn et al. 2009); the first component (PC1) from a principal components analysis had a positive loading for all measures, reflecting a combined measure of activity levels and propensity to explore novel objects and areas. Square root transformation led to an approximately normal distribution, and this was used as the measure of exploratory behaviour. We ignored repeat assays and did not correct for within-seasonal temporal variation, as reported previously (Quinn et al. 2009), because all assays were undertaken at the same stage of the season.

Immediately following the EB assay, the response of each bird to a startle stimulus was assessed by instigating the sudden movement of a stick from the corner of the novel environment room, controlled remotely using a length of colourless line fed under the door. This was carried out between 2 and 4 min after the end of the EB assay, when the bird was on either of the two trees closest to the stimulus stick. This trait could not be assessed for a number of individuals ($n=26$) who did not land on either of these two trees within 4 min of the end of the EB assay.

Assays were recorded using a video camera (Handycam, DCR-SR33E, camcorder, Sony, UK) and videos analysed subsequently to determine (1) whether each bird reacted to the movement of the stick by leaving the tree and (2) the time taken to react to the movement of the stick, defined as the time taken between the start of the movement stimulus and the bird's feet leaving the tree (± 0.05 s). Examination of the startle response times showed a clear bimodal distribution, with birds either reacting immediately to the movement of the stick, or reacting to the sound of the stick hitting the floor (or not reacting at all). Thus, birds were split into those that either did or did not react to the initial movement of the startle stimulus (defined as leaving the perch before the noise of the stick hitting the floor was audible), which was used as the response variable.

Parasite screening

DNA was extracted from blood samples using a standard ammonium acetate method and screened for parasites using the protocols of Waldenström et al. (2004) for detection of infection by *Plasmodium* spp., and Hellgren et al. (2004), for detection of infection by *Leucocytozoon* spp. The presence of *Plasmodium* was established using primers HaemNF and HaemNR2 nested within HaemF and HaemR2 (Waldenström et al. 2004), and *Leucocytozoon* spp. were detected using primers HaemFL and HaemR2L nested within primers HaemNFI and HaemNR3 (Hellgren et al. 2004). Reactions for detection of *Plasmodium* were carried out in a working volume of 25 μ l containing 50–200 ng template DNA, 1.25 mM dNTPs, 3 mM $MgCl_2$, 0.4 μ M of each primer, 1 $\times$ GoTaq Flexi Buffer (Promega, Madison, WI) and 1 U GoTaq Flexi (Promega, Madison, WI); the protocol for detection of *Leucocytozoon* differed only in that the concentration of $MgCl_2$ was 1.5 mM. Multiple positive controls of DNA from birds with known infections and negative controls containing deionised water in place of DNA were included on each plate to ensure successful amplification of target DNA and lack of contamination respectively. The protocol of Waldenström et al. (2004) also detects *Haemoproteus* spp., however this parasite is known to occur at a very low prevalence of $\sim 0.8\%$ at our study site (Cosgrove et al. 2008) and thus we assumed that all infections detected using this protocol were *Plasmodium*.

The PCR protocol for first round reactions consisted of a denaturation step of 94°C for 3 min followed by 20 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 45 s, with a terminal extension step of 72°C for 10 min; the protocol for second round reactions contained 35 cycles but otherwise consisted of an identical thermal profile. PCR reactions were carried out on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California).

Statistical analysis

Statistical analyses were carried out in R (R Core Development Team 2009). Associations between behavioural variables were established using a χ^2 test (for the binary variables, problem-solving performance and startle response) and two binomial generalised linear models (for associations between exploratory behaviour and each of the two binary variables).

Three generalised linear models were used to determine whether parasite infection status was associated with problem-solving performance, exploration behaviour or startle response. Capture and natal areas can also be associated with behavioural variation in our population (Quinn et al., unpublished data) and the likelihood of parasitism (Wood et al. 2007). We therefore controlled for any effects of these two factors on all three response variables and the likelihood of parasite infection separately. Where these terms were found to influence a behavioural variable they were included in further analysis (full model results are provided in Appendices 1 and 2 in the Electronic supplementary material).

All models were fitted with parasite infection status, age, sex and year as main effects. To determine whether behavioural responses to parasitism differed between years, age classes or sexes, we also fitted the appropriate two-way interactions. The startle response model also included the distance of the bird from the startle stimulus to control for any effects that proximity to the stimulus may have on the response. We also repeated these tests for effects of each parasite family separately.

Model comparisons using AIC values were used to determine whether terms significantly improved the fit of the model; those that did not were removed in a stepwise fashion until only those terms that improved the fit of the model at $\alpha=10\%$ remained. Following model simplification, each non-significant main effect was reinserted into the minimum adequate model (MAM) in turn and compared with the MAM using AIC comparisons and likelihood ratio tests at $\alpha=10\%$ to ensure lack of association with the response variable and to validate the robustness of the MAM. Although model simplification by stepwise deletion has been criticised in the literature (Whittingham et al. 2006; Mundry and Nunn 2009), a recent study validated stepwise deletion as a method of model selection and established that it performed just as well as other methods of producing predictive models (Murtaugh 2009). In addition, simplification of our models made no difference to the terms considered to significantly influence the response variables.

The interactions between infection status and sex were significant for all three behavioural traits. Post hoc analyses were subsequently conducted on each sex separately to

establish whether opposing trends were present in both sexes or whether a significant trend was present in only one sex and not in the other. The structure of models used in these analyses was identical to those used previously, but without the main effect of sex.

To determine whether any associations were also found with the number of parasite infections a bird had, the above three models were rerun, substituting the parasitized or not parasitized term with the number of parasite infections (0, 1 or 2).

Results

Infection rates and behavioural correlations

In total, 59 male and 32 female great tits were assayed during October 2008 and 2009. No sex biases were found in infection rates, with 56% of females infected with at least one parasite, compared with 61% of males; 40% of females and 41% of males were infected by *Plasmodium* spp., and 38% of females and 39% of males were infected by *Leucocytozoon* spp. Older birds were more likely to be infected ($\chi^2=6.80$, $p=0.009$), but infection rate was not significantly influenced by natal area (GLM, $F=0.86$, $p=0.54$) or capture site (GLM, $F=0.06$, $p=0.94$) in our sample.

Problem-solving performance (PSP) was not significantly linked to EB (GLM, $t_{1,88}=1.46$, $p=0.14$), a result that we also report elsewhere using a much larger sample size (Cole et al. 2011) and therefore these two behavioural traits are independent of one another. In contrast startle response was not independent of the other two variables because it correlated, although not significantly, with both PSP ($\chi^2=3.31$, $p=0.07$) and EB (GLM, $t_{1,62}=1.80$, $p=0.07$), with problem solvers and faster explorers both more likely, although not significantly, to show a startle response.

Problem-solving performance

The likelihood of a bird solving a problem overnight did not differ significantly between the sexes (males, 46% solved and females, 50% solved), but was predicted by an interaction between sex and whether a bird was infected (GLM, $z_{1,86}=3.56$, $p=0.0004$). Male birds were more likely to solve if infected (GLM, $z_{1,57}=3.27$, $p=0.001$; Fig. 1a), whereas infected females were less likely to solve (GLM, $z_{1,29}=-3.12$, $p=0.002$; Fig. 1a). We also note that amongst uninfected birds, females were far more likely to solve problems than males (Fig. 1a). The proportions of birds solving also differed between years (GLM, $z_{1,87}=2.65$, $p=0.008$), with 60.5% solving in 2008 compared with only 37.3% solving in 2009. Age did not significantly

influence the likelihood of solving (GLM, $LRT=0.07$, $p=0.80$) and there was no evidence for the effects of infection differing between age classes (age $\times$ infection, $LRT=0.01$, $p=0.92$) or between years (year $\times$ infection, $LRT=0.05$, $p=0.82$).

Sex differences in associations were also found when the analyses were carried out for each parasite family separately (*Plasmodium* spp.: GLM, $z_{1,86}=2.21$, $p=0.04$, Fig. 1b; *Leucocytozoon* spp.: GLM, $z_{1,86}=2.93$, $p=0.003$, Fig. 1c; for full model details see Table 1 and Online Appendix 1 in the Electronic supplementary material). An interaction between the number of parasite infections and sex also influenced problem-solving performance (GLM, $z_{1,86}=3.24$, $p=0.001$), with a higher percentage of solvers with an increasing number of infections in males but no linear trend in females (Fig. 2a; full model details are given in Table 2).

Exploratory behaviour

Exploration behaviour did not differ significantly between the sexes (males, 0.95 ± 0.06 and females, 1.01 ± 0.07), but was predicted by an interaction between sex and parasite infection status (GLM, $F_{1,86}=4.16$, $p=0.05$). No evidence was found for a relationship between male exploration behaviour and infection status (GLM, $F_1=0.14$, $p=0.71$; Fig. 1d); however infected females were more exploratory (GLM, $F_{1,29}=6.18$, $p=0.02$; Fig. 1d). Adults were more exploratory than juveniles (GLM, $F_{1,87}=8.89$, $p=0.004$; juvenile, 0.86 ± 0.04 and adult, 1.17 ± 0.09). Exploration behaviour did not differ significantly between years (GLM, $F_1=0.69$, $p=0.41$), and there was no evidence for the effects of infection differing between years (year $\times$ parasite, $F_1=0.28$, $p=0.60$) or between age classes (age $\times$ infection, $F_1=1.73$, $p=0.19$). Sex differences in associations were also found when examining associations with *Plasmodium* spp., although this was not significant for *Leucocytozoon* spp. (*Plasmodium* spp.: GLM, $F_{1,81}=4.35$, $p=0.04$, Fig. 1e; *Leucocytozoon* spp.: GLM, $F_{1,81}=2.97$, $p=0.09$, Fig. 1f; for full model details see Table 1 and Online Appendix 1 in the Electronic supplementary material). Exploratory behaviour was also influenced by an interaction between sex and the number of parasite infections (GLM, $F_{1,81}=4.39$, $p=0.04$), with exploration in females increasing with a higher number of infections (Fig. 2b; full model details are given in Table 2).

Startle response

Whether or not a bird responded to a sudden movement did not differ significantly between the sexes (males, 85% reacted and females, 79% reacted), but was predicted by an interaction between sex and infection status (GLM, $z_{1,59}=$

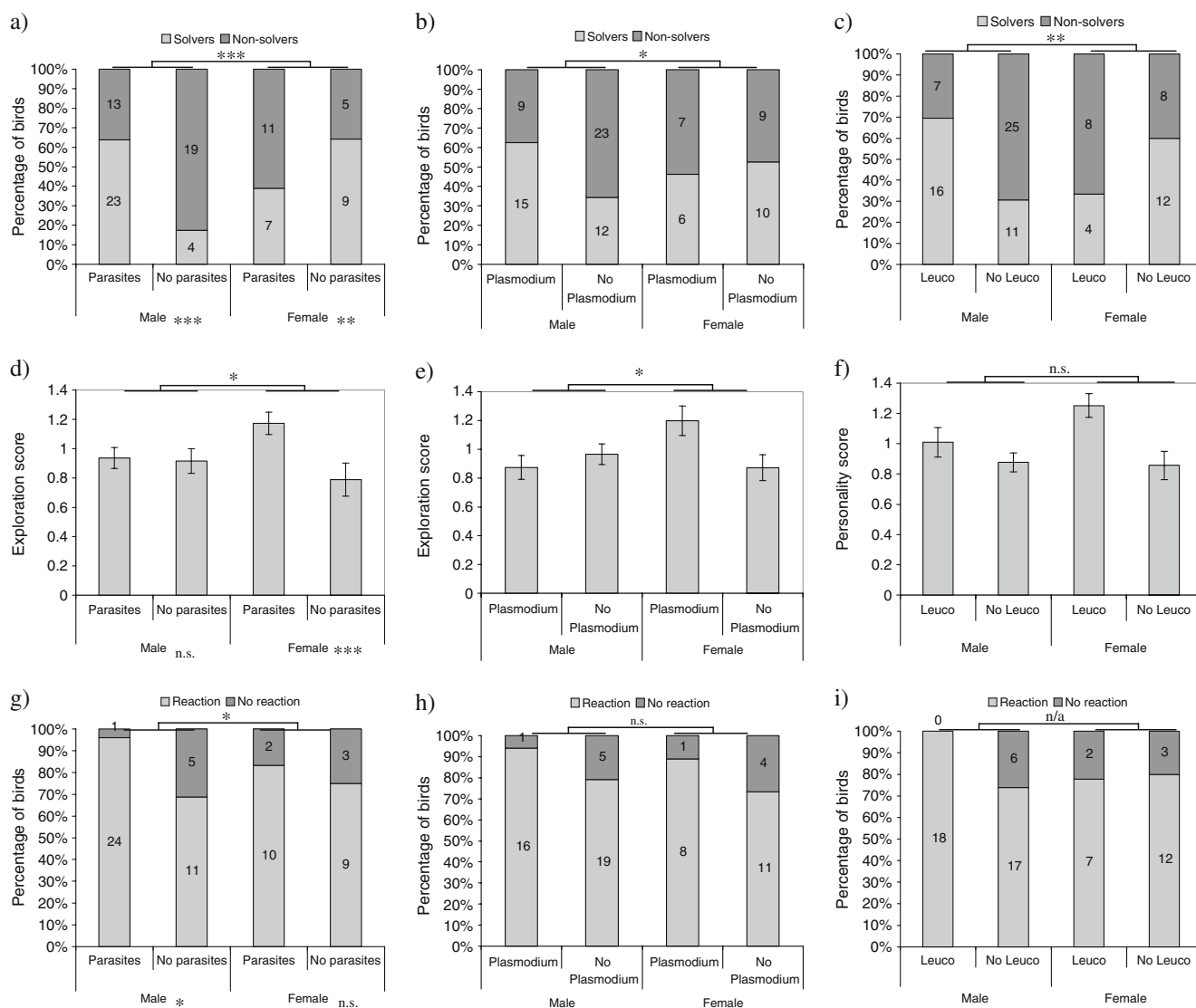


Fig. 1 Associations between **a–c** problem-solving performance, **d–f** exploration behaviour and **g–i** startle response and **a, d** and **g** infection by either parasite family, **b, e** and **h** infection by *Plasmodium* spp. and **c, f** and **i** infection by *Leucocytozoon* spp. **a–c** and **g–i** Bars show percentage of total number of birds; numbers in bars show sample size. **d–f** Bars show mean $\pm$ 1 SE. Lines above bars represent

significance levels for the sex $\times$ infection interaction; symbols by the sexes indicate behavioural associations with parasitism within each sex: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Full results from statistical analyses for (**a, d** and **g**) are shown in Table 1; results of analyses for (**b, c, e, f, h** and **i**) are given in the [Electronic supplementary material](#)

2.03, $p = 0.04$). The likelihood of a female reacting was not significantly associated with infection status (GLM, LRT = 0.03 $p = 0.87$); however males were more likely to react if infected (GLM, $z_{1, 38} = 2.46$, $p = 0.01$; Fig. 1g). Whether a bird responded also varied with year (GLM, $z_{1, 60} = -2.86$, $p = 0.004$), with more birds reacting in 2008 (97%) than in 2009 (74%), and with the distance of the bird from the startle stimulus (GLM, $z_{1, 61} = -2.39$, $p = 0.02$); birds that did not react were on average further away from the startle stimulus than those that did (did react, 1.50 ± 0.06 m and did not react, 1.72 ± 0.06 m). Age was not significantly associated with the likelihood of reacting (GLM, LRT =

0.95, $p = 0.33$), and there was no evidence of the effects for parasites differing with age (age $\times$ parasite, LRT = 0.98, $p = 0.32$), or between years (year $\times$ infection, LRT = 0.60, $p = 0.44$). Sex differences in associations were not found when examining associations with *Plasmodium* spp. (GLM, $z_{1, 85} = 0.38$, $p = 0.54$, Fig. 1h; for full model details see Table 1 and Online Appendix 1 in the Electronic supplementary material); associations with *Leucocytozoon* spp. could not be tested as the inclusion of the interaction term destabilised the model. An interaction between sex and the number of parasite infections also influenced the likelihood of birds responding to a startle stimulus (GLM, $Z_{1, 59} = 2.18$, $p =$

Table 1 Full model results from general linear models analysing associations between parasite infection and each of problem-solving performance (PSP), exploration behaviour (EB) and startle response (SR) for all birds; males only and females only

Variable	Parasite infection						Males						Females					
	<i>df</i>	<i>z/LRT</i>	<i>F</i>	<i>p</i>	Estimate	SE	<i>df</i>	<i>z/LRT</i>	<i>F</i>	<i>p</i>	Estimate	SE	<i>df</i>	<i>z/LRT</i>	<i>F</i>	<i>p</i>	Estimate	SE
PSP																		
Sex (male)	1, 89	2.299		0.002	-2.587	0.851												
Parasites (yes)	1, 88	-3.041		0.046	-1.634	0.820	1, 57	3.273		0.001	2.129	0.650	1, 29	-3.121		0.002	-1.040	0.738
Year (2009)	1, 87	-2.648		0.008	-1.412	0.533	1	1.002		0.317			1, 20	-2.500		0.012	-1.887	0.790
Age	1	0.065		0.799			1	0.017		0.897			1	1.099		0.294		
Sex×parasites	1, 86	3.566		0.0004	4.024	1.128												
Year×parasites	1	0.054		0.816			1	1.268		0.260			1	0.001		0.999		
Age×parasites	1	0.011		0.915			1	0.001		0.999			1	0.001		0.999		
EB																		
Sex (male)	1, 85		0.631	0.429	0.183	0.131												
Parasites (yes)	1, 86		3.063	0.084	0.292	0.137	1		0.140	0.710			1, 29		6.183	0.019	0.284	0.114
Year (2009)	1		0.685	0.410			1, 54		4.105	0.048	-0.222	0.110	1		0.628	0.435		
Age (adult)	1, 84		8.888	0.004	0.247	0.095	1		0.553	0.460			1, 30		18.929	0.0002	0.433	0.120
Capture site (Great Wood)	2, 82		3.182	0.047	-0.278	0.127	2		0.335	0.717			2		0.242	0.786		
Capture site (Marley)					-0.192	0.154												
Sex×parasites	1, 81		4.156	0.045	-0.354	0.174												
Year×parasites	1		0.284	0.595			1		0.001	0.989			1		1.264	0.272		
Age×parasites	1		1.725	0.193			1		2.102	0.154			1		1.148	0.295		
SR																		
Sex (male)	1, 63	-1.170		0.242	-1.386	1.185												
Parasites (yes)	1, 62	0.120		0.904	0.153	0.153	1, 38	2.459		0.014	3.766	1.531	1	0.029		0.866		
Year (2009)	1, 60	-2.859		0.004	-3.824	1.337	1, 37	-2.009		0.045	-3.071	1.529	^b					
Age	1	0.951		0.329			1	1.514		0.219			1	1.702		0.192		
Distance	1, 61	-2.386		0.017	-0.038	0.016	1, 39	-1.567		0.117 ^a	-0.034	0.021	1	1.513		0.219		
Sex×parasites	1, 59	2.031		0.042	3.988	1.964												
Year×parasites	1	0.603		0.437			1	0.129		0.719			^b					
Age×parasites	1	0.984		0.321			1	0.523		0.470			1	1.009		0.315		

Estimates±1 SE are presented for all terms remaining in the minimum adequate model (MAM); terms considered to significantly influence the response variable are highlighted. Statistics presented for non-significant terms are from model comparisons using either likelihood ratio tests (binomial) or *F* tests (Gaussian), depending on the error distribution of the model

^a This term showed marginal significance in influencing the fit of the model ($LRT_1=3.63$, $p=0.06$) and thus remained in the MAM but is not considered to significantly influence the response variable

^b The inclusion of this term and its interaction destabilised the model (probably due to all females reacting in 2008); thus, we had to omit this term from the maximal model and did not consider it any further

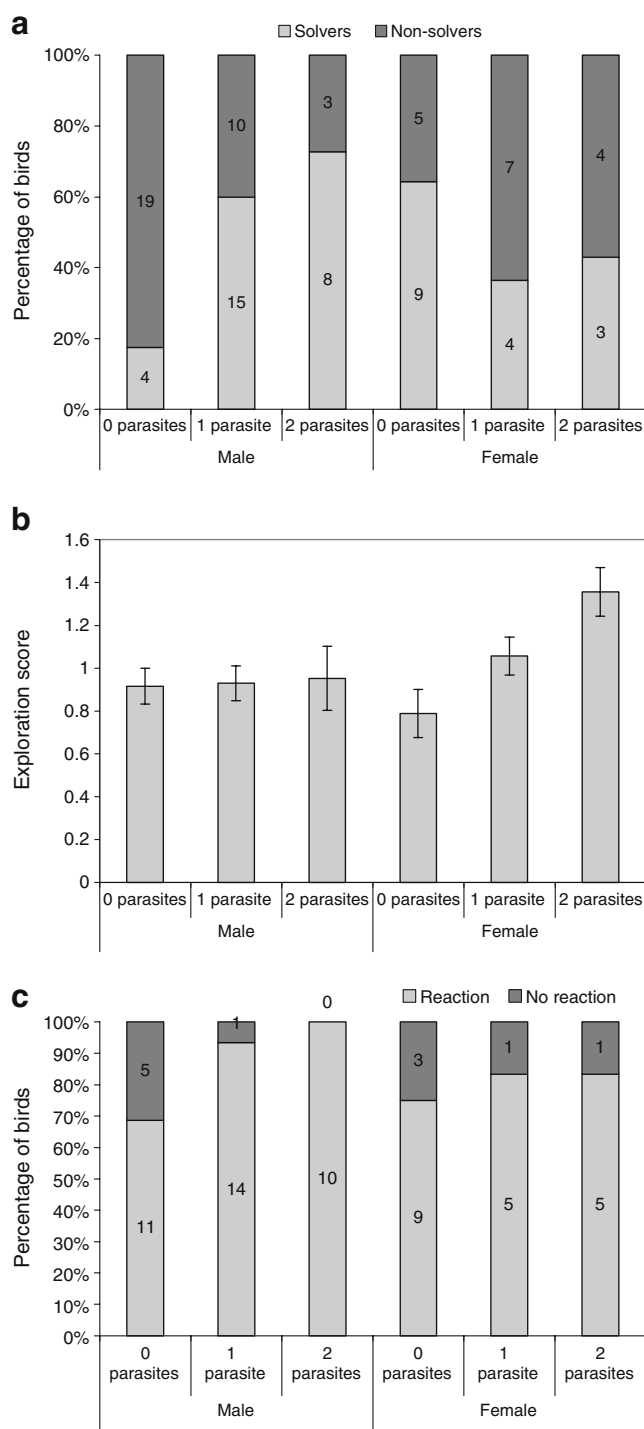


Fig. 2 Associations between the number of parasite infections and **a** problem-solving performance, **b** exploration behaviour and **c** startle response. **a**, **c** Bars show percentage of total number of birds; numbers in bars show sample size. **b** Bars show mean $\pm$ 1 SE. Full results for statistical analyses are shown in Table 2

0.03): males with one parasite were more likely to respond than uninfected birds and all males infected with two parasites reacted to the stimulus (Fig. 2c; full model details are given in Table 2).

Table 2 Full model results from general linear models analysing associations between the number of parasite infections and each of problem-solving performance (PSP), exploration behaviour (EB) and startle response (SR) for all birds

Variable	PSP				EB				SR			
	df	z/LRT	p	Estimate	SE	df	F	p	Estimate	SE	df	p
Sex (male)	1, 89	-2.68	0.007	-1.947	0.727	1, 85	0.504	0.480	0.173	0.124	1, 63	0.210
No. parasites	1, 88	-1.561	0.119	-0.795	0.509	1, 86	3.390	0.069	0.205	0.088	1, 62	0.971
Year (2009)	1, 87	-2.474	0.013	-1.238	0.501	1, 80	0.687	0.410	0.233	0.096	1, 60	0.004
Age	1, 89	0.024	0.876	NA	NA	1, 84	8.901	0.004	-0.301	0.126	1, 59	0.262
Capture site (Great Wood)	NA	NA	NA	NA	NA	2, 82	3.421	0.037	-0.194	0.154	NA	NA
Capture site (Marley)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Distance	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sex $\times$ no. parasites	1, 86	3.242	0.001	2.300	0.708	1, 81	4.390	0.039	-0.234	0.112	1, 61	0.017
Year $\times$ no. parasites	1, 84	2.698	0.101	NA	NA	1, 79	0.813	0.370	NA	NA	1, 59	0.029
Age $\times$ no. parasites	1, 85	0.717	0.397	NA	NA	1, 78	0.738	0.393	NA	NA	1, 57	0.439
											1, 56	0.524

NA refers to terms that were not included in the model

Discussion

We report several associations between three behavioural traits and infection by two avian malaria parasites. Elsewhere, we show that two of these behavioural traits, exploration behaviour (Quinn et al. 2009) and problem-solving performance (Cole et al. 2011), are repeatable or heritable, while variables similar to startle response are also known to be repeatable (van Oers et al. 2004). On average there was no difference in any of the behaviours or in infection rate between the sexes, but the association between behaviour and parasitism was strongly sex dependent.

Problem solving

Firstly, we found that malaria infection was associated with higher problem-solving performance in male great tits, agreeing with another study that found a positive relationship between avian malaria parasite prevalence and feeding innovations at the species level (Garamzegi et al. 2007). The effect reported in Garamzegi et al. (2007) is assumed to be behaviourally mediated because problem solvers often have more diverse foraging habits which should increase their exposure to parasites (Garamzegi et al. 2007). We speculate that this is also the case for male great tits because in our population problem solving amongst males is associated with a greater diet breadth (Cole et al., unpublished data). Furthermore, the sexes are also known to have different foraging strategies in winter when the majority of birds found turning over leaves in search of beechmast on the ground are male, while females are more commonly seen foraging for invertebrates in the trees. Sex differences in foraging strategies at times of the year when malaria vectors are active are also likely to occur (Gosler 1987). The association in males could be the result of adaptive parasite mediation but we are unaware of any examples involving a vector-transmitted parasite. In contrast to the pattern we found for males, avian malaria was associated with lower problem solving amongst female great tits. Kihara et al. (2006) found a negative association between malaria and cognition in humans but do not mention sex differences, and the directly transmitted protozoan parasite *Crithidia bombi* has also been linked to a parasite mediated reduction in spatial memory among female bumblebees (Gegear et al. 2006). Finally, we note that whilst there was no difference in either exploration behaviour or startle response between uninfected males and females, uninfected females were four times more likely to problem solve than uninfected males, a pattern that is only seen when infection status is taken into account. Thus, controlling for important sources of environmental variation, such as parasitism, may be crucial when exploring

latent sex differences in behavioural variation (e.g. Healy et al. 2009).

Exploration behaviour

We found that female great tits with malaria infection were faster explorers than uninfected females. Associations between activity levels and parasitism are relatively widely reported across trophically, directly and vector-transmitted parasites (summarised in Table 3.7 of Moore 2002); however, the direction of the associations varies. The majority of parasites are associated with lethargy, probably due to parasite load or competition by the parasite for host resources (Edelaar et al. 2003), but some trophically transmitted parasites are associated with increased movement, for example rats infected by *Toxoplasma gondii* show increased activity, which improves the chance of the parasite being transmitted through predation of the intermediate host (Webster 1994). Wilson et al. (1993) suggested that differences in directly transmitted gut parasite fauna between bold and shy pumpkinseed sunfish *Lepomis gibbosus* were caused by differential habitat use by bold and shy personality types, that is, the associations were behaviourally rather than parasite mediated. More recent studies found positive associations between exploratory behaviour, or boldness, and directly transmitted parasites such as tick load or viral infection (Natoli et al. 2005; Easterbrook et al. 2007; Boyer et al. 2010), and Boyer et al. (2010) concluded that tick load resulted from space use. Boyer et al. (2010) also found sex differences in both parasite load and in exploratory behaviour, with adult males more active than both females and juveniles, and males having a higher parasite load. Similar to our results, Webster (1994) found female rats infected by congenitally acquired and trophically transmitted *Toxoplasmosis gondii* to be more active than uninfected females, but found no association in males (Webster 1994), though no explanation was offered for these sex differences. As the prevalence of infection varies on a large spatial scale at Wytham, (Wood et al. 2007) and the parasites in our system are vector transmitted, it seems plausible that this spatial variation, or perhaps variation at a finer micro-habitat scale, explains the association between higher exploration behaviour and malaria among female great tits in our study; territoriality restricting space use by males during the breeding season when vectors are more likely to cause infection may explain the lack of association seen in males.

To conclude this section, we note that exploration behaviour and other temperament traits are currently the focus of considerable attention among evolutionary ecologists because, amongst other reasons, they are easily measured under standardized conditions, making them ideal candidate behaviours for quantitative genetic studies.

Previous quantitative genetic analysis in our population showed that the ‘permanent environment’ component of variation was equally, if not more, important than additive genetic variance (Quinn et al. 2009). If the association we report here is parasite mediated, malaria may be an important contributor to these permanent environment effects.

Startle latency

We also found links between parasite infection and startle latency. Empirical data offer conflicting evidence for whether parasites have a negative or positive effect on anti-predation behaviour and predation risk, and to our knowledge no study has considered sex differences in these associations (Valkiunas 2005; Holmstad et al. 2006; Møller and Nielsen 2007; Médoc et al. 2009). Some trophically transmitted parasites influence host anti-predator behaviour, either to avoid falling prey to a non-host predator (Médoc et al. 2009) or to increase the likelihood of predation and transmission to the parasite’s target host (Webster 1994). Avian malaria is not a trophically transmitted parasite and our finding that infected males were more likely to respond to startle latency supports the hypothesis of a parasite-mediated anti-predator response in order to increase transmission rate through continued exposure to vectors. However, comparative analyses suggest that species with higher vector-transmitted blood parasite prevalence have a higher risk of predation (Møller and Nielsen 2007). Two studies show that avian malaria parasites specifically are associated with a reduction in the likelihood of fleeing from a stimulus (Valkiunas 2005; Holmstad et al. 2006). However, interpreting the functional significance of fleeing versus the use of alternative anti-predation measures, for example crypsis, is fraught with difficulty and can depend on the predator species involved (Cresswell 1993) or on an individual’s state (Cresswell 1994), a point that has previously been made in the context of personality (Quinn and Cresswell 2005). Previous studies have assumed that fleeing was the adaptive response stimulus (Valkiunas 2005; Holmstad et al. 2006) but freezing could be adaptive under some circumstances (Quinn and Cresswell 2005), and therefore the effect observed could feasibly be an adaptive parasite-mediated effect on the host.

Sex dependence

All of the associations we reported between behaviour and parasite infection were sex dependent. Sex differences in behavioural traits associated with latent toxoplasmosis in humans are thought to be mediated by hormone levels altering dopamine levels (Lindová et al. 2006; Flegr et al. 2008). In this case, female hormones protect the doper-

minergic system, causing less severe, or even opposing behavioural effects among infected women when compared with those shown by infected men (Lindová et al. 2006). Sex-specific hormonal effects could be behind at least some of the sex differences that we observed. However, whether hormones play a role in the patterns observed says nothing about whether the associations are parasite or behaviourally mediated. Hormones are a likely cause of male biases in infection, as testosterone is known to decrease immune function (Oppliger et al. 2004), and birds with higher plasma testosterone levels tend to have a higher intensity of malaria infection (Saino et al. 1995). The intensity of infection could potentially indicate whether our associations are parasite mediated: other systems have demonstrated quantitative impacts of parasites upon behaviour (e.g. Lafferty and Morris 1996) with the more extreme behaviour observed when parasite load is at its highest and the parasite has reached its transmissible stage (Koella et al. 2002; Lacroix et al. 2005; Lefèvre and Thomas 2008; Parker et al. 2009) and in theory quantitative PCR methods could be used to investigate causality in our system. However, the intensity of avian malaria infection is also strongly dependent upon individual immune response (Zehindjiev et al. 2008) and thus, prior knowledge of individual immune status, along with the relative status of infection within an individual would be required before this approach could allow inference into the direction of causality. This is also likely to be confounded by the strong association between physiological and behavioural traits (Koolhaas 2008; Coppens et al. 2010). Additionally, PCR does not allow discrimination between transmissible and non-transmissible stages of the parasite and thus cannot discriminate between stages that should be associated with behavioural response if the behavioural association is parasite mediated (Koella et al. 2002).

Multiple infections

We found associations between an increased number of infections and male (although not female) problem-solving performance, exploration behaviour in females, and startle response in males, with doubly infected birds exhibiting more extreme behaviour. Co-infection can have additive, synergistic or protective effects on hosts (Read and Taylor 2001; Pedersen and Fenton 2006; Helmby 2009) frequently through immunomodulated interactions within the host (Niikura et al. 2008; Putaporntip et al. 2010; van Duivenvoorde et al. 2010), although the impacts of co-infection on behavioural variation or parasite manipulation have seldom been explored (Thomas et al. 2002; Rigaud et al. 2010). A study in a similar system to ours suggested that the avian immune system promotes multiple infections within the same erythrocyte, providing a direct means for interaction of multiple parasites within the

same host cell (Martínez-de la Puente et al. 2007). Interestingly, Martínez-de la Puente et al. (2007) suggests that this effect is amplified in females due to increased levels of immunoglobulin. This warrants further investigation within our population as to whether parasites interact within a host to influence host behaviour or whether host behaviour influences the likelihood of multiple infections.

Morphological and life-history associations with parasitism can be host–parasite specific and differ between parasite species (e.g. Rätti et al. 1993). On the one hand, this suggests that the similarity in the effects of both parasites on our behavioural traits points to behaviourally mediated effects, especially since both are transmitted by blood-sucking dipterans (Valkiunas 2005). On the other hand, even if parasites do modify behaviour, then they are likely to initiate a feedback loop to increase the likelihood of transmission (e.g. Brown et al. 2002) or reciprocal effects may occur (Blanchet et al. 2009), and thus experimental work, or work involving the recapture and resampling of marked individuals (e.g. Beldomenico et al. 2009), may be the only way to clarify the causality of these relationships, although investigating correlations between the intensity of the infective stage of the parasite and behaviour of wild-caught individuals would complement this approach. An alternative explanation is that some of the patterns observed could arise because susceptibility to parasite-mediated mortality may vary between behavioural phenotypes (Barber and Dingemanse 2010) because of correlated differences, either in immunocompetence (Zehindjiev et al. 2008; Réale et al. 2010), or in the ability to compensate for the negative effects of parasitism on behaviour through differing sensitivity to stress (Réale et al. 2010).

Conclusions

Our results provide evidence that avian malaria can be associated with multiple, repeatable and partially independent behavioural traits at the same time within individual hosts. Previous studies have tested for associations between parasite infection and multiple behavioural traits but in most cases the traits were similar to one another, or associations were found with a restricted number of behavioural traits (Boyer et al. 2010; Coats et al. 2010). Problem-solving performance (Keagy et al. 2009), exploration behaviour (Dingemanse et al. 2002; Both et al. 2005; Quinn et al. 2009) and startle latency (e.g. van Oers et al. 2004; Quinn and Cresswell 2006) are functionally significant traits and therefore the implications of the associations between avian malaria and behaviour are likely to be far reaching within our population. Elsewhere, it has been suggested that effect specificity might lead to the decoupling of correlations between behavioural traits if the

selective consequences on behaviour as a result of parasitism are sufficiently strong (Barber and Dingemanse 2010). Our results emphasise an alternative process, which is that the selective consequences of parasitism might act on multiple behavioural traits independently of one another. Another conclusion from our results is that latent sexual differences in behavioural variation might be observed only after controlling for the effects of parasitism, as differences in problem-solving performance are only seen in uninfected birds where females were almost four times as likely to problem solve than uninfected males. Finally, although only an experimental approach can confirm the direction of causality, we suspect the associations are primarily behaviourally mediated in our population, given the likely link between at least two of these behavioural traits and exposure to vectors. If true, this highlights the importance of controlling for the effects of exposure to parasites in evolutionary ecological studies of behavioural variation, which could lead to complex effects when multiple behavioural traits are correlated within individuals (Sih et al. 2004a). Alternatively, if the effects prove to be primarily parasite mediated, traditional host–parasite studies in natural populations should consider that the effects of parasites on behaviour are potentially broader than might otherwise have been assumed.

Ethical standards

The authors declare that all work described in this manuscript complies with the laws of the UK. Behavioural assays and temporary maintenance of wild birds in captivity was carried out under licence from Natural England. Blood sampling was carried out under licence from the Home Office.

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Conflicts of interest The authors declare that they have no conflicts of interest.

References

- Barber I, Dingemanse NJ (2010) Parasitism and the evolutionary ecology of animal personality. *Phil Trans R Soc B* 365:4077–4088
- Beldomenico PM, Telfer S, Lukomski L, Gebert S, Bennett M, Begon M (2009) Host condition and individual risk of cowpox virus infection in natural animal populations: cause or effect? *Epidemiol Infect* 137:1295–1301

- Blanchet S, Thomas F, Loot G (2009) Reciprocal effects between host phenotype and pathogens: new insights from an old problem. *Trends Parasitol* 25:364–369
- Both C, Dingemanse NJ, Drent PJ, Tinbergen JM (2005) Pairs of extreme avian personalities have highest reproductive success. *J Anim Ecol* 74:667–674
- Boyer N, Réale D, Marmet J, Pisanu B, Chapuis J-L (2010) Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. *J Anim Ecol* 79:538–547
- Brown SP, Loot G, Teriokhin A, Brunel A, Brunel C, Guégan JF (2002) Host manipulation by *Ligula intestinalis*: a cause or consequence of parasite aggregation? *Int J Parasitol* 32:817–824
- Coats J, Poulin R, Nakagawa S (2010) The consequences of parasitic infections for host behavioural correlations and repeatability. *Behav* 147:367–382
- Cole EF, Cram DL, Quinn JL (2011) Individual variation in spontaneous problem-solving performance among wild great tits. *Anim Behav* 81:491–498
- Coppens CM, De Boer SF, Koolhaas JM (2010) Coping styles and behavioural flexibility: towards underlying mechanisms. *Phil Trans R Soc* 365:4021–4028
- Cosgrove CL, Wood MJ, Day KP, Sheldon BC (2008) Seasonal variation in *Plasmodium* prevalence in a population of blue tits *Cyanistes caeruleus*. *J Anim Ecol* 77:540–548
- Cresswell W (1993) Escape responses by redshanks, *Tringa totanus*, on attack by avian predators. *Anim Behav* 46:609–611
- Cresswell W (1994) Song as a pursuit-deterrent signal, and its occurrence relative to other anti-predation behaviours of skylark (*Alauda arvensis*) on attack by merlins (*Falco columbarius*). *Behav Ecol Sociobiol* 34:217–223
- Dingemanse NJ, Both C, Drent PJ, Van Oers K, Van Noordwijk AJ (2002) Repeatability and heritability of exploratory behaviour in great tits from the wild. *Anim Behav* 64:929–938
- Dufva R (1996) Blood parasites, health, reproductive success, and egg volume in female great tits *Parus major*. *J Avian Biol* 27:83–87
- Easterbrook JD, Kaplan JB, Glass GE, Pletnikov MV, Klein SL (2007) Elevated testosterone and reduced 5-HIAA concentrations are associated with wounding and hantavirus infection in male Norway rats. *Horm Behav* 52:474–481
- Edelaar P, Drent J, De Goeij P (2003) A double test of the parasite manipulation hypothesis in a burrowing bivalve. *Oecologia* 134:66–71
- Flegr J, Lindová J, Kodým P (2008) Sex-dependent toxoplasmosis-associated differences in testosterone concentration in humans. *Parasitology* 135:427–431
- Garamzegi LZ, Erritzoe J, Moller AP (2007) Feeding innovations and parasitism in birds. *Biol J Linn Soc* 90:441–455
- Gegeer RJ, Otterstatter MC, Thomson JD (2006) Bumble-bee foragers infected by a gut parasite have an impaired ability to utilize floral information. *Proc R Soc Lond B* 273:1073–1078
- Gosler A (1987) Pattern and process in the bill morphology of the great tit *Parus major*. *Ibis* 129:451–476
- Gosling SD, John OP (1999) Personality dimensions in nonhuman animals: a cross-species review. *Curr Dir Psychol Sci* 8:69–75
- Greenberg R (2003) The role of neophobia and neophilia in the development of innovative behaviour. In: Reader SM, Laland KN (eds) *Animal innovation*. Oxford University Press, Oxford, pp 175–196
- Hart BL (1990) Behavioral adaptations to pathogens and parasites—5 strategies. *Neurosci Biobehav Rev* 14:273–294
- Healy SD, Bacon IE, Haggis O, Harris AP, Kelley LA (2009) Explanations for variation in cognitive ability: behavioural ecology meets comparative cognition. *Behav Processes* 80:288–294
- Hellgren O, Waldenström J, Bensch S (2004) A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. *J Parasitol* 90:797–802
- Helmby H (2009) Gastrointestinal nematode infection exacerbates malaria-induced liver pathology. *J Immunol* 182:5663–5671
- Holmstad PR, Jensen KH, Skorping A (2006) Vector-borne parasites decrease host mobility: a field test of freeze or flee behaviour of willow ptarmigan. *Int J Parasitol* 36:735–740
- Keagy J, Savard J-F, Borgia G (2009) Male satin bowerbird problem-solving ability predicts mating success. *Anim Behav* 78:809–817
- Kihara M, Carter JA, Newton CRJC (2006). The effect of *Plasmodium falciparum* on cognition: a systematic review. *Trop Med Int Health* 11:386–397
- Knowles SCL, Palinauskas V, Sheldon BC (2010) Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *J Evol Biol* 23:557–569
- Koella JC, Rieu L, Paul REL (2002) Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. *Behav Ecol* 13:816–820
- Koolhaas JM (2008) Coping style and immunity in animals: making sense of individual variation. *Brain Behav Immun* 22:662–667
- Lacroix R, Mukabana WR, Gouagna LC, Koella JC (2005) Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biol* 3:e298
- Lafferty KD, Morris AK (1996) Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology* 77:1390–1397
- Laland KN, Reader SM (1999) Foraging innovation in the guppy. *Anim Behav* 57:331–340
- Lefèvre T, Adamo SA, Biron DG, Misse D, Hughes D, Thomas F (2009) Invasion of the body snatchers: the diversity and evolution of manipulative strategies in host-parasite interactions. *Adv Parasitol* 68:45
- Lefèvre T, Roche B, Poulin R, Hurd H, Renaud F, Thomas F (2008) Exploiting host compensatory responses: the 'must' of manipulation? *Trends Parasitol* 24:435–439
- Lefèvre T, Thomas F (2008) Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases. *Infect Genet Evol* 8:504–519
- Lindová J, Novotná M, Havlíček J, Jozífková E, Skallová A, Kolbeková P, Hodný Z, Kodým P, Flegr J (2006) Gender differences in behavioural changes induced by latent toxoplasmosis. *Int J Parasitol* 36:1485–1492
- Martínez-de la Puente J, Merino S, Tomás G, Moreno J, Morales J, Lobato E, García-Fraile S (2007) Can the host immune system promote multiple invasions of erythrocytes *in vivo*? Differential effects of medication and host sex in a wild malaria-like model. *Parasitology* 134:651–655
- Médoc V, Rigaud T, Bollache L, Beisel JN (2009) A manipulative parasite increasing an antipredator response decreases its vulnerability to a nonhost predator. *Anim Behav* 77:1235–1241
- Merino S, Moreno J, Sanz JJ, Arriero E (2000) Are avian blood parasites pathogenic in the wild? A medication experiment in blue tits (*Parus caeruleus*). *Proc R Soc Lond B* 267:2507–2510
- Møller AP, De Lope F, Saino N (2004) Parasitism, immunity, and arrival date in a migratory bird, the barn swallow. *Ecology* 85:206–219
- Møller AP, Nielsen JT (2007) Malaria and risk of predation: a comparative study of birds. *Ecology* 88:871–881
- Moore J (2002) *Parasites and the behavior of animals*. Oxford University Press, Oxford
- Mundry R, Nunn CL (2009) Stepwise model fitting and statistical inference: turning noise into signal pollution. *Am Nat* 173:119–123
- Murtaugh PA (2009) Performance of several variable-selection methods applied to real ecological data. *Ecol Lett* 12:1061–1068
- Natoli E, Say L, Cafazzo S, Bonanni R, Schmid M, Pontier D (2005) Bold attitude makes male urban feral domestic cats more

- vulnerable to feline immunodeficiency virus. *Neurosci Biobehav Rev* 29:151–157
- Niikura M, Kamiya S, Kita K, Kobayashi F (2008) Coinfection with nonlethal murine malaria parasites suppresses pathogenesis caused by *Plasmodium berghei* NK65. *J Immunol* 180:6877–6884
- Oppliger A, Giorgi MS, Conelli A, Nembrini M, John-Alder HB (2004) Effect of testosterone on immunocompetence, parasite load, and metabolism in the common wall lizard (*Podarcis muralis*). *Can J Zool* 82:1713–1719
- Parker GA, Ball MA, Chubb JC, Hammerschmidt K, Milinski M (2009) When should a trophically transmitted parasite manipulate its host? *Evolution* 63:448–458
- Pedersen AB, Fenton A (2006) Emphasizing the ecology in parasite community ecology. *Trends Ecol Evol* 22:133–139
- Putaporntip C, Jongwutiwes S, Thongaree S, Seethamchai S, Grynberg P, Hughes AL (2010) Ecology of malaria parasites infecting Southeast Asian macaques: evidence from cytochrome *b* sequences. *Mol Ecol* 19:3466–3476
- Quinn JL, Cresswell W (2005) Personality, anti-predation behaviour and behavioural plasticity in the chaffinch *Fringilla coelebs*. *Behaviour* 142:1377–1402
- Quinn JL, Cresswell W (2006) Testing domains of danger in the selfish herd: sparrowhawks target widely spaced redshanks in flocks. *Proc R Soc Lond B* 273:2521–2526
- Quinn JL, Patrick SC, Bouwhuis S, Wilkin TA, Sheldon BC (2009) Heterogeneous selection on a heritable temperament trait in a variable environment. *J Anim Ecol* 78:1203–1215
- R Core Development Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available at: <http://www.R-project.org>
- Rätti O, Dufva R, Alatalo RV (1993) Blood parasites and male fitness in the pied flycatcher. *Oecologia* 96:410–414
- Read AF, Taylor LH (2001) The ecology of genetically diverse infections. *Science* 292:1099–1102
- Réale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO (2010) Personality and the emergence of the pace-of-life syndrome at the population level. *Phil Trans R Soc B* 365:4051–4063
- Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. *Biol Rev* 82:291–318
- Rigaud T, Perrot-Minnot M-J, Brown MJF (2010) Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc R Soc Lond B* 277:3693–3702
- Roth G, Dicke U (2005) Evolution of the brain and intelligence. *Trends Cognit Sci* 9:250–257
- Saino N, Möller AP, Bolzern AM (1995) Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav Ecol* 6:397–404
- Sanchez MI, Ponton F, Schmidt-Rhaesa A, Hughes DP, Misse D, Thomas F (2008) Two steps to suicide in crickets harbouring hairworms. *Anim Behav* 76:1621–1624
- Sih A, Bell A, Johnson JC (2004a) Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol* 19:372–378
- Sih A, Bell AM, Johnson JC (2004b) Behavioral syndromes: an integrative overview. *Q Rev Biol* 79:241–277
- Svensson L (1992) Identification guide to European passerines. British Trust for Ornithology, Thetford
- Thomas F, Fauchier J, Lafferty KD (2002) Conflict of interest between a nematode and a trematode in an amphipod host: test of the "sabotage" hypothesis. *Behav Ecol Sociobiol* 51:296–301
- Valkiunas G (2005) Avian malaria parasites and other haemosporidia. CRC Press, Boca Raton
- Van Duivenvoorde LM, Van der Voorberg-Wel A, Van der Werff NM, Braskamp G, Remarque EJ, Kondova I, Kocken CHM, Thomas AW (2010) Suppression of *Plasmodium cynmolgi* in rhesus macaques by coinfection with *Babesia microti*. *Infect Immun* 78:1032–1039
- Van Oers K, Drent PJ, De Goede P, van Noordwijk AJ (2004) Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. *Proc R Soc Lond B* 271:65–73
- Van Riper III C, Van Riper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecol Monogr* 56:327–344
- Verbeek MEM, Drent PJ, Wiepkema PR (1994) Consistent individual differences in early exploratory-behavior of male great tits. *Anim Behav* 48:1113–1121
- Waldenström J, Bensch S, Hasselquist D, Ostman O (2004) A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. *J Parasitol* 90:191–194
- Webster JP (1994) The effect of *Toxoplasma gondii* and other parasites on activity levels in wild and hybrid *Rattus norvegicus*. *Parasitology* 109:583–589
- Webster JP (2001) Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. *Microbiol Inform* 3:1037–1045
- Webster SJ, Lefebvre L (2001) Problem solving and neophobia in a columbiform-passeriform assemblage in Barbados. *Anim Behav* 62:23–32
- Whittingham MJ, Stephens PA, Bradbury RB, Freckleton RP (2006) Why do we still use stepwise modelling in ecology and behaviour? *J Anim Ecol* 75:1182–1189
- Wilson DS, Coleman K, Clark AB, Biederman L (1993) Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. *J Comp Psychol* 107:250–260
- Wood MJ, Cosgrove CL, Wilkin TA, Knowles SCL, Day KP, Sheldon BC (2007) Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Mol Ecol* 16(15):3263–3273
- Zehntindjiev P, Ilieva M, Westerdahl H, Hansson B, Valkiunas G, Bensch S (2008) Dynamics of parasitaemia of malaria parasites in a naturally and experimentally infected migratory songbird, the great reed warbler *Acrocephalus arundinaceus*. *Exp Parasitol* 119:99–110